UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

September 26, 2011

MEMORANDUM

Subject: Response to Protocol Review for 84542-PA1, Cupron Enhanced Hard Surfaces as a Supplement to Sanitization; No DP Barcode Assigned

From: Tajah L. Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Emily Mitchell, Chief
Product Science Branch
Antimicrobials Division (7510P)

To: Jacqueline Campbell PM34/Stacey Grisby
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: CUPRON, INC.
800 East Leigh Street, Suite 123
Richmond, VA 23219

Formulation from the Label: revised Label does not include formulation statement

I BACKGROUND

Through the current submission, Cupron is addressing questions from the Agency's protocol review (dated July 1, 2011). The current submission also includes a revised protocol for Agency review. The proposed protocol is based on an accepted Agency protocol, and this review is for surface equivalency only.

II USE DIRECTIONS

Directions on the proposed label provided the following instructions for the preparation and use of the product:

The use of Antimicrobial Cupron Enhanced Hard Surface does not replace standard infection control procedures and good hygienic practice. Antimicrobial Cupron Enhanced Hard Surface must be cleaned and sanitized according to standard practice. Health care facilities must maintain the product in accordance with infection control guidelines; users must continue to follow all current infection control practices, including those practices related to disinfection of environmental surfaces. In order for the antimicrobial Cupron Enhanced Hard Surface have proper antimicrobial effect, the product must be cleaned and maintained according to the directions included on this label.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Test Requirements for Efficacy of Copper Enhanced Hard Surfaces as a Supplement to Sanitization

Sanitizer efficacy testing must be conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048), before additional organisms or claims (residual self-sanitizing activity and continuous reduction) are considered. Acceptable efficacy testing is required against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048) as a non-food contact sanitizer before additional microorganisms or claims can be granted. For claims of Continuous Reduction and/or Residual Self-Sanitizing Activity, initial efficacy testing against *Staphylococcus aureus* and *Enterobacter aerogenes* is required before additional microorganisms are granted. To support a supplemental sanitization claim on a copper alloy surface, a 99.9% reduction in numbers of the test organism(s) be obtained as compared to the carrier quantitation control. Efficacy data can support the claim "kills greater than 99.9% of bacteria* within two hours (* Includes list of tested organisms). Claims are limited to indoor, hard, non-porous surfaces where cleaning practices are consistent. The following language is required on the registered products, the use of a copper surface is a supplement to and not a substitute for standard infection control practices; user must continue to follow all current infection control practices, including those practices related to cleaning and disinfection of environmental surfaces. The copper surface material has been shown to reduce microbial contamination, but does not necessarily prevent cross contamination.

Proper Care and Use of Antimicrobial Copper Alloys: The use of copper surfaces does not replace standard infection control procedures and good hygienic practices. Antimicrobial copper surfaces must be cleaned and sanitized according to standard practice. Heath care facilities must maintain the product in accordance with infection

control guidelines; users must continue to follow all current infection control practices, including those practices related to disinfection of environmental surfaces.

Cleaning Directions: Routine cleaning to remove dirt and filth is necessary for good sanitization and to assure the effective antibacterial performance of the Antimicrobial Copper Alloy surface. Cleaning agents typically used for traditional touching surfaces are permissible; the appropriate cleaning agent depends on the type of soiling and the measure of sanitization required.

This product must not be waxed, painted, lacquered, varnished, or otherwise coated.

IV REGISTRANT'S RESPONSES TO AGENY'S INITIAL PROTOCOL REVIEW

Agency's Initial Response 1. The registrant must explain the distribution of Cupron when impregnated with Corian or other polymers. The registrant must include detailed information regarding additional polymers for which Cupron will be impregnated. Additionally, test carriers must represent all proposed polymer blends. Furthermore, efficacy data must be generated using both non-pigmented and pigmented carrier types.
Registrant's Response: The distribution of Cupron within the hard surface polymer is homogenously distributed throughout the active area as shown in the figures provided. The Figure 1 corresponds to an area on the hard surface and the Figure 2 is the EDS mapping of the same area. The purple dots in the Figure 2 represent copper which is uniformly distributed in the polymer. The Cupron impregnated surfaces will be constructed of only one polymer blend. The single polymer Cupron will be incorporated into will be a polyester isophthalic and acrylic ester blended resin. That exact blend will then be tested. Cupron understands that any other polymer blend must be specifically tested. Cupron assumes that any pigment will be acceptable for nontested pigments (in other words a test on a blue colored polymer slab will constitute efficacy for a red colored slab of the same material). Please note Corian is a registered trademark and Cupron is not authorized to use this mark.
Agency's Follow-up Response: No additional information is required.

Agency's Initial Response 2. The registrant stated in the submitted letter (dated February 21, 2010), the use sites are "similar to Corian". Corian is limited to kitchen countertops, bathroom vanity tops, and wall cladding in showers. Several of the proposed use sites are not typically composed of Corian or Corian-liked polymers. The registrant must review the label, and remove or provide a rationale for those sites that are not composed of Corian or Corian-liked polymers.
Registrant's Response: As noted in response 1, the items claimed on the label are all composed of only one polymer blend. That blend will be tested with this protocol. Please note Corian is a registered trademark and Cupron is not authorized to use this mark. The manufacturing process (cast or injection molding) used to produce solid products from polyester isophthalic and acrylic ester blended resins are capable of forming flat surface products such as counter tops and table tops and more complex shaped objects such as sinks, tubs and rails amongst others.
Agency's Follow-up Response: No additional information is required

Agency's Initial Response 3. Food contact surfaces are not acceptable use sites for this protocol.
Registrant's Response: All surfaces on the label that potentially could be food contact surfaces have been specified to be non-food contact surfaces. The abstract of the protocol specifically states nonfood contact surfaces.
Agency's Follow-up Response: No additional information is required.

Agency's Initial Response 4. Sanitizer efficacy testing must be conducted against *Staphylococcus aureus* (ATCC# 6538) and *Enterobacter aerogenes* (ATCC# 13048), before additional organisms or claims (residual self-sanitizing activity and continuous reduction) can be considered. For claims of Continuous Reduction and/or Residual Self-Sanitizing Activity, initial efficacy testing against *Staphylococcus aureus* and *Enterobacter aerogenes* is required before additional microorganisms are granted.
Registrant's Response: Sanitizer efficacy data will be demonstrated against *Staphylococcus aureus* (ATCC# 6538) and *Enterobacter aerogenes* (ATCC# 13048), before additional organisms or claims will be pursued.
Agency's Follow-up Response: No additional information is required.

V SYNOPSIS OF SUBMITTED PROTOCOL

Protocol Title: Test Method for Efficacy of Copper Alloy Surfaces as a Sanitizer; MRID Number was not assigned

Test Organisms:	*Staphylococcus aureus* (ATCC 6538) *Enterobacter aerogenes* (ATCC 13048)
Additional Test Organisms:	*Pseudomonas aeruginosa* (ATCC 15442) Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) *Escherichia coli* O157:H7 (ATCC 35150)

Test System

Carrier Surfaces and Preparation: Cut the Cupron Enhanced Hard surface into individual 1" x 1" square carriers. Identical hard surfaces without Cupron carriers (1" x 1") must be incorporated into the test system. Cupron Enhanced Hard Surfaces will be utilized as the test carriers and identical Hard Surfaces without Cupron surfaces control carriers for this assay. Clean carriers with alcohol, rinse with deionized water, and allow to air dry. Sterilize carriers prior to use in test. After sterilization, place each carrier into a plastic Petri dish matted with two pieces of filter paper using sterile forceps. Test five (5) test carriers per material per organism.

Preparation of Test Organisms:
Staphylococcus aureus, Pseudomonas aeruginosa, Methicillin Resistant *Staphylococcus aureus*: From stock cultures, inoculate tubes of the appropriate broth with organism, and incubate for 24±2 hours at 35-37°C. Using a 4-mm inside diameter disposable sterile plastic transfer loop, transfer at least three consecutive daily cultures in appropriate broth prior to use as inoculum. Transfer two (2) loopfuls of culture into 10 ml broth medium. Transfers more than 15 days away from stock culture should not be used for

the inocula for this test. Use 48±4 hour cultures on for the inocula on the day of testing. On the day of use, aspirate pellicle from the *Pseudomonas aeruginosa* culture.

Enterobacter aerogenes: From stock cultures, inoculate tubes of Tryptic Soy Broth and incubate for 24±2 hours of 25-30°C. Using a 4-mm inside diameter disposable sterile plastic transfer loop, perform at least three consecutive daily transfers of cultures in Tryptic Soy Broth prior to use as inoculum. Transfer two (2) loopfuls of culture into 10 ml broth medium. Transfers more than 15 days away from stock culture should not used for the inocula for this test.

For each test organism, thoroughly mix the culture on a "vortex" mixer and allow to settle. Aspirate the upper two thirds of this suspension and use as the inoculum for testing. Add an organic soil load containing Triton X-100 (to aid in spreading the inoculum) to the test culture.

Addition of Organic Soil Load: Add 0.25 ml aliquot of serum + 0.05 ml Triton X-100 to 4.70 ml bacteria suspension to yield a 5% fetal bovine serum and 0.01% Triton X-100 soil load.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing is required when utilizing a resistant organism. On the day of testing, verify the antimicrobial resistance pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA). Subculture the organism onto a Tryptic Soy agar (TSA) plates (or 5% sheep blood agar plate (BAP)), and incubate for approximately 24 hours at 35-37°C. Following incubation, make a suspension of the test organism equal to 0.5 McFarland Standard in 0.85% sterile saline. Streak the suspension onto Mueller Hinton agar. Place an oxacillin disc in the center of the inoculated Mueller Hinton plate. Invert and incubate for ≥ 24 hours at 35-37°C. Following incubation, measure the zone of inhibition using a calibrated caliper. Concurrently run *Staphylococcus aureus* (ATCC 25923), a control organism, with the test organism to confirm the validity of the assay. The interpretation of the zone of inhibition is based on established National Committee for Clinical Laboratory Standards (NCCLS) performance standards.

Inoculation of Carriers: Inoculate each sterile carrier at staggered intervals with 0.02 ml of 48±4 hour culture using a calibrated pipettor. Spread the inoculum to within 1/8 inch of the edges of the carrier. Dry carriers at ambient conditions for 20-40 minutes with lids ajar. The exposure period begins immediately after drying.

Neutralization and Subculture: Following the 120 minute exposure period, transfer carriers to 20 ml of the appropriate neutralizer solution at staggered intervals. Sonicate each neutralizer jar after five minutes to suspend any survivors from the carriers, and rotate to mix. Within one hour after sonicating the carriers, prepare serial dilutions (10^{-1} - 10^{-4}) of the neutralized solution from each of the jars and plate in duplicate for survivors using standard spread plate technique and Tryptic Soy agar (TSA) plates (or 5% sheep blood agar plates (BAP)).

Incubation and Observation: Incubate the plates at 35-37°C for 48±4 hours prior to observation for number of colonies. Incubate *E. aerogenes* plates at 25-30°C for 48±4 hours prior to observation for number of colonies. Following incubation, visually enumerate the plates.

Study Controls

Purity Controls: Perform a "streak plate for isolation" on each organism culture and following incubation examine in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control: Culture, incubate, and visually examine the serum used for soil load. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control: Add a representative uninoculated test and control carrier to the neutralizing subculture medium. Incubate and examine for growth the subculture medium containing each carrier. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control: Incubate and visually examine a representative sample of uninoculated neutralizing subculture medium. The acceptance criterion for this study control is lack of growth.

Viability Control: Add a representative inoculated control carrier to the subculture medium. Incubate and visually examine the subculture medium containing the carrier for growth. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control: Perform the neutralization confirmation control to demonstrate the neutralizer's ability to inactivate the test carrier. The neutralization of the test carriers is confirmed by using sterile test carriers and neutralizing as in the test procedure. A 1.0 ml aliquot of a diluted suspension of the test organism yielding ≤100 CFU/ml of neutralizing subculture medium is transferred to the jar and mixed. A 1.0 ml aliquot of this mixed solution is plated in duplicate. A numbers control is performed utilizing sterile stainless steel control carriers. The resulting plates are incubated as in the test and enumerated. The acceptance criterion for this study is growth within 1 $\log_{10}$ of the numbers control.

Inoculum Count: Serial dilutions of the cultures used as the inocula are prepared and plated. Tryptic Soy Agar (or 5% Sheep Blood agar) plates should be used for all organisms. Incubate the resulting plates for 48±4 hours at 35-37°C (for *E. aerogenes* 25-30°C), and then count the colonies to determine the number of organisms per milliliter of inoculum present at the start of the test.

Carrier Quantitation Control: Use three (3) inoculated Hard Surfaces without Cupron control carriers to determine the number of test organisms per carrier per time point. Transfer the control carriers to neutralizing subculture media and sonicate as in the test. Prepare ten-fold serial dilutions of the neutralizing subculture medium and plate 1.0 ml of the appropriate dilutions in duplicate to yield countable numbers. Incubate and enumerate the plates as in the test. The acceptance criterion for this study control is a minimum geometric mean of 2.0×10^4 CFU/carrier.

Study Acceptance Criteria

Test Substance Performance Criteria: To support a supplemental sanitization claim on a copper alloy surface, a 99.9% reduction in numbers of the test organism(s) be obtained as compared to the carrier quantitation control.

Control Acceptance Criteria: The study controls must perform according to the criteria detailed in the study controls description section.

Data Analysis

Calculations

Number of Organisms Surviving per Carrier

$$\text{CFU/carrier} = \frac{(\text{average number colonies/plate @ dilution}) \times (\text{dilution factor}) \times (\text{volume neutralized solution})}{(\text{volume plated})}$$

The carrier population was calculated and reported using data from the most appropriate dilution(s).

Geometric Mean Number of Organisms Surviving on Control Carrier

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_3}{3}$$

Where X equals CFU/control carrier

Geometric Mean of Number of Organisms Surviving on Test Carrier

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}Y_1 + \text{Log}_{10}Y_2 + \text{Log}_{10}Y_3 + \text{Log}_{10}Y_4 + \text{Log}_{10}Y_5}{5}$$

Where Y equals CFU/test carrier

Percent Reduction

% reduction = [(a-b) / a] x 100

Where:

a= geometric mean of the number of organisms surviving on the inoculated control carriers

b= geometric mean of the number of organisms surviving on the test carriers.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Test Results)

Used to calculate the neutralization confirmation control

Label Claims Supported by the Protocol

This surface kills greater than 99.9% of bacteria* within two hours.
*Includes list of tested organisms.

V CONCLUSION AND LABEL RECOMMENDATION

1. The submitted protocol, "Test Method for Efficacy of Cupron Enhanced Hard Surfaces as a Supplemental sanitizer" is acceptable. The registrant may initiate testing using the approved protocol. The registrant must include the exact type of control carriers identified as "hard surfaces without Cupron" in the protocol. As a condition of registration, a stewardship program, as described on the Agency accepted protocol website, must be included when efficacy data is submitted.